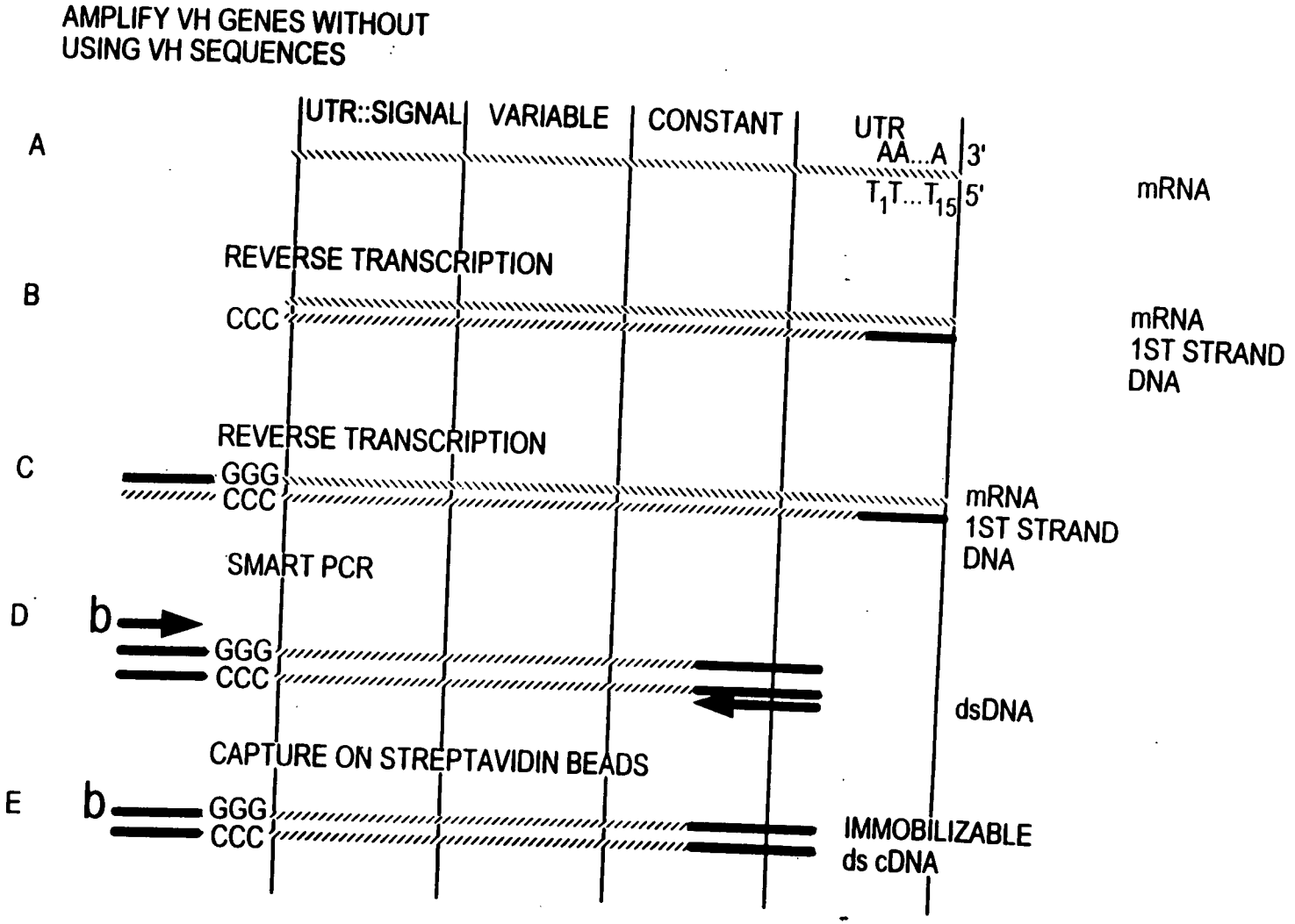


FIG. 1

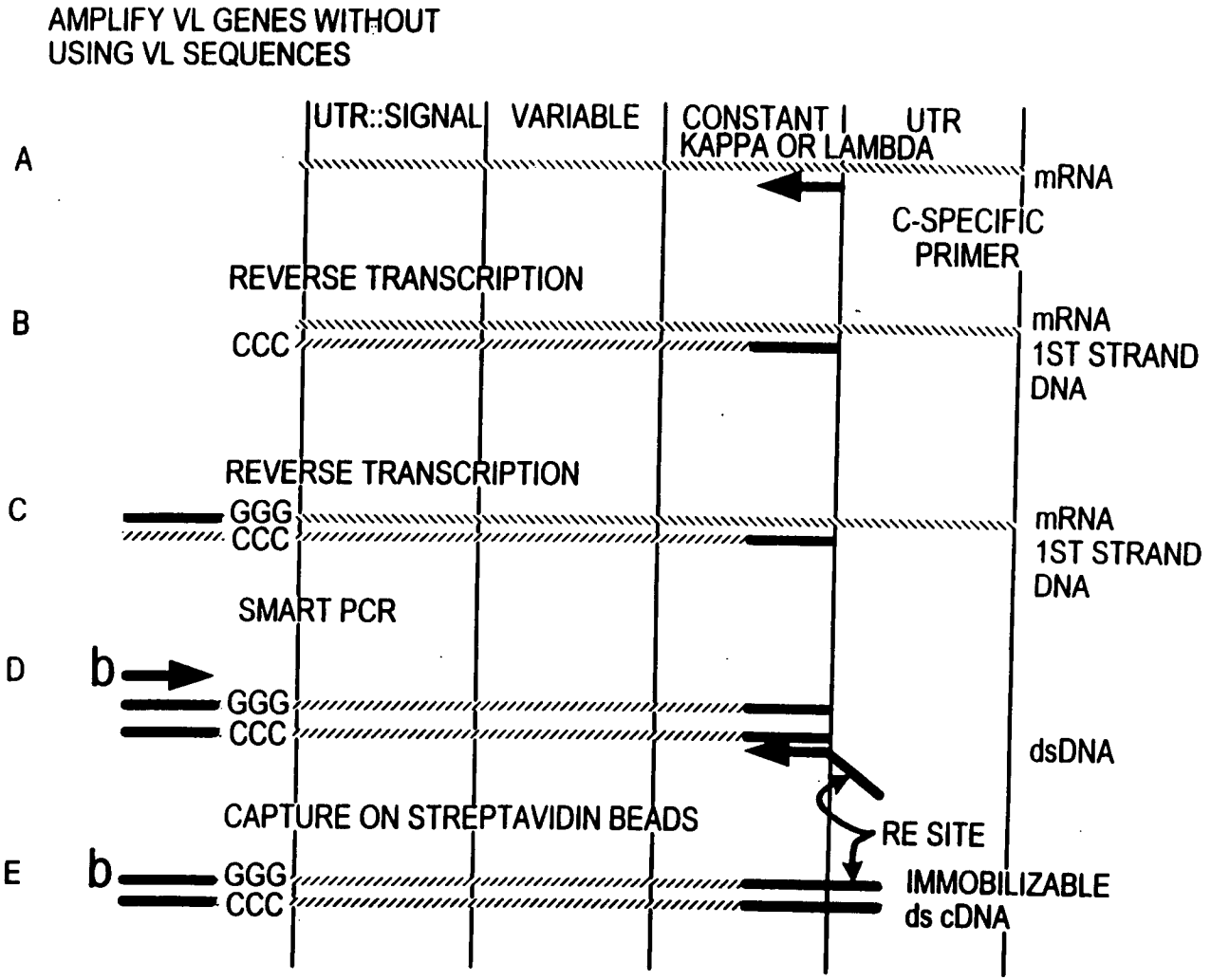


FIG. 2

RACE non-biased antibody V-gene amplification

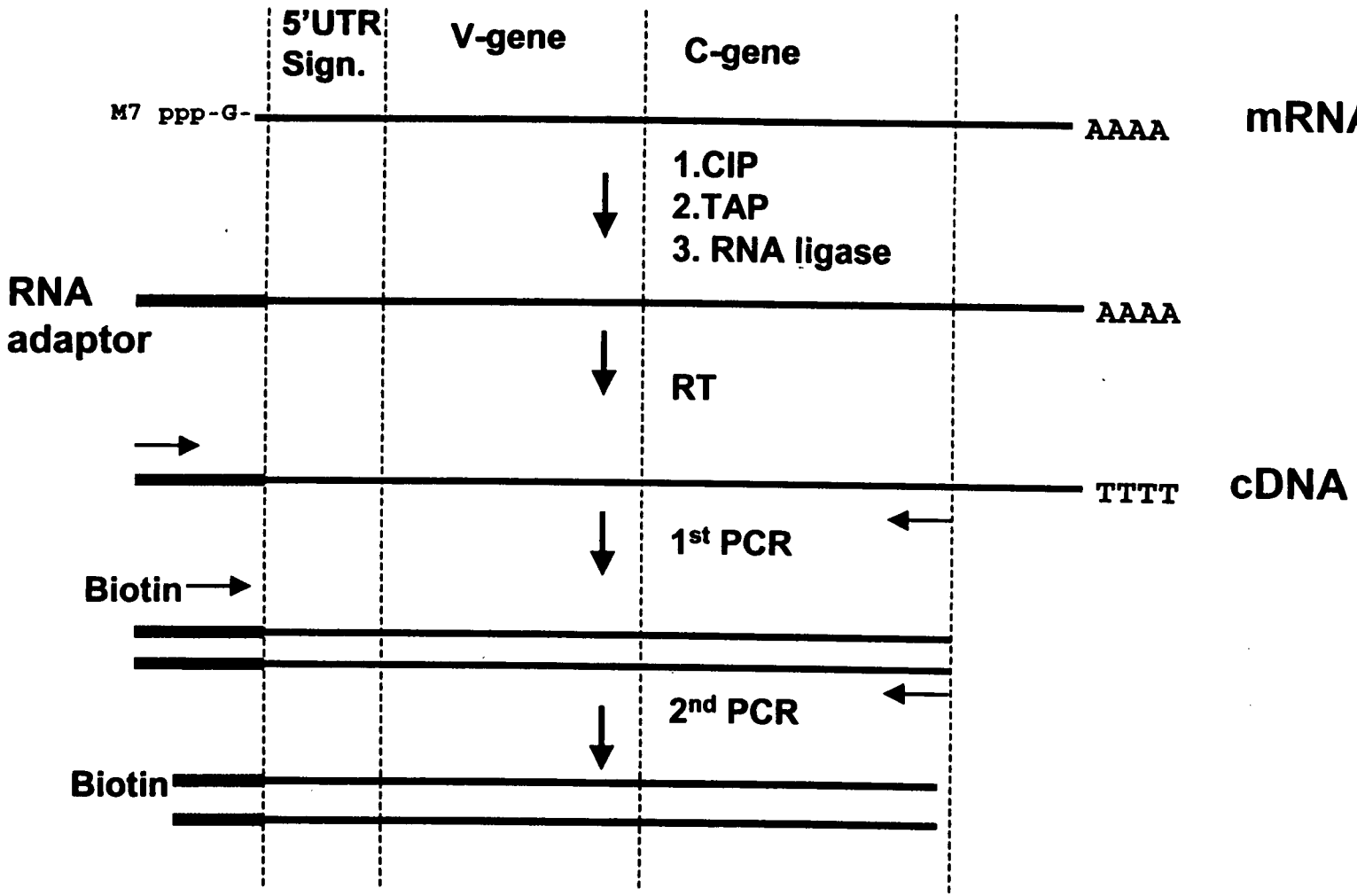
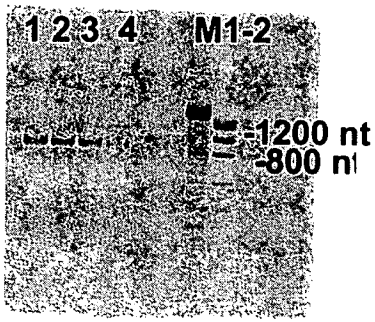


FIG. 3

1, 2, 3 and 4 are patient samples

1st PCR heavy chains



1st PCR light chains

Kappa

Lambda

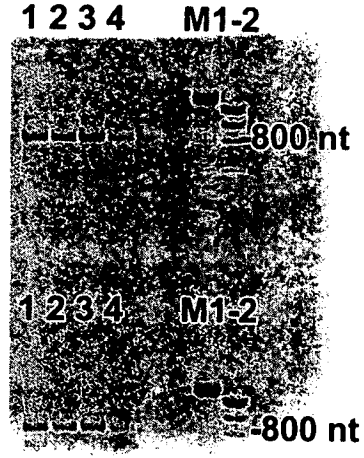
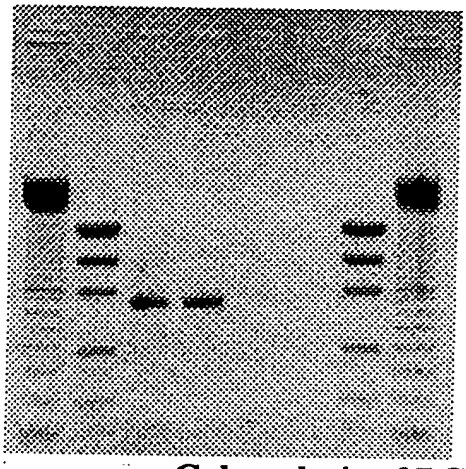


FIG. 4

1 2 3 4 5 6 7 8



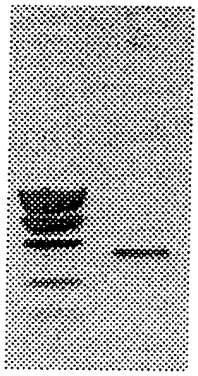
Gel analysis of PCR product from extender-kappa amplification

Approx. 75ng/5µl → 15ng/µl

1 - 100bp
2 - LDM
3 - 50ng template
4 - 10ng template
5 - ssDNA unligated
6 - negative control
7 - LDM
8 - 100bp

FIG. 5

1 2



Gel purified PCR product from extender-kappa amplification

Concentration : ± 35ng/µl

1 - LDM
2 - 1µl purif.

FIG. 6

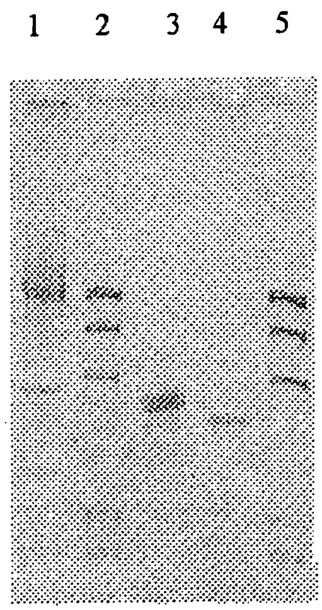
1 2 3 4 5 6 7

Gel-analysis of digested κ-ssDNA

1µl digested ssDNA ≈ 8ng ssDNA
Total volume of 50µl = 400ng ssDNA
➔ 400ng ssDNA available for ligation of the bridge-extenders

1 - 100bp
2 - LDM
3 - 1µl ssDNA pure
4 - 4µl beads after dig.
5 - 8µl beads after dig.
6 - LDM
7 - 100bp

FIG. 7



Gel analysis of extender – cleaved kappa ligation

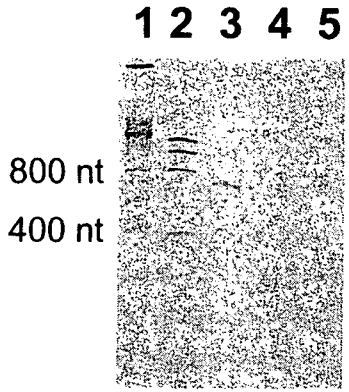
20ng/5μl eluted material → 4ng/μl

1- 100bp
2 - LDM
3 - Ligationmix, 4μl
4 - Unligated ssDNA
5 - LDM

FIG. 8

Cleavage and ligation Kappa light chains

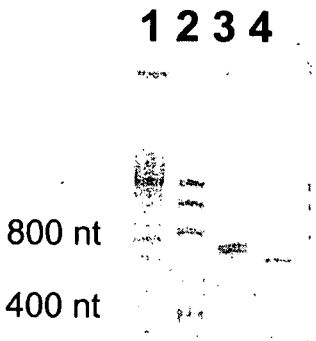
A) BsmA1 cleavage



1. 100 bp marker
2. LDM marker
3. Sup. ssDNA after dig.
4. beads after dig. (uncleaved material)
5. DNA before cleavage

80% cleavage

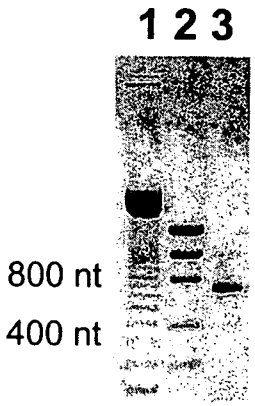
B) Bridge Ligation



1. 100 bp marker
2. LDM marker
3. Ligationmix
4. Unligated ssDNA

90% ligation

C) PCR



1. 100 bp marker
2. LDM marker
3. 50 ng template (13 cycles)

FIG. 9

VH-CDR1
1 Y 1 M 1

VH-CDR2
2 I 2 3 S G G 1 T 1 YADSVKG

FIG. 10

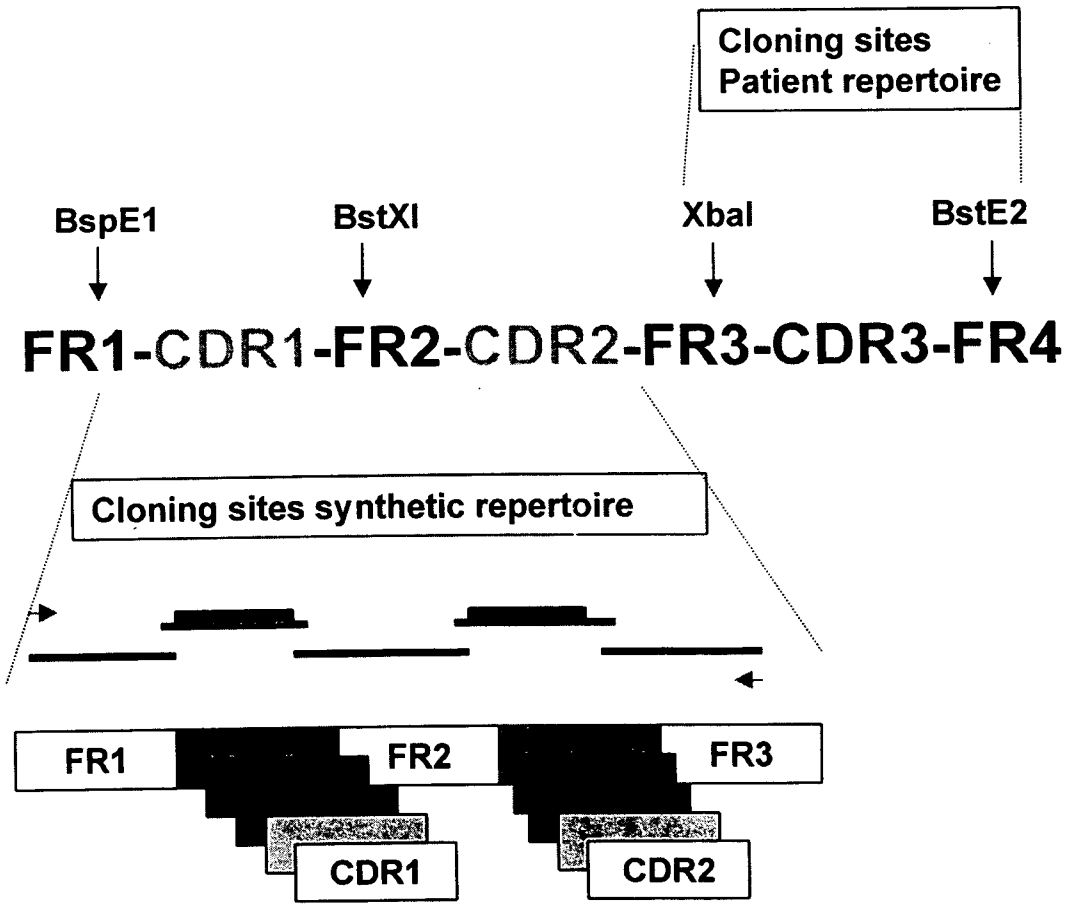

Cloning sites
Patient repertoire
BspE1
BstXI
XbaI
BstE2
FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4
Cloning sites synthetic repertoire
FR1
FR2
FR3
CDR1
CDR2

FIG. 11

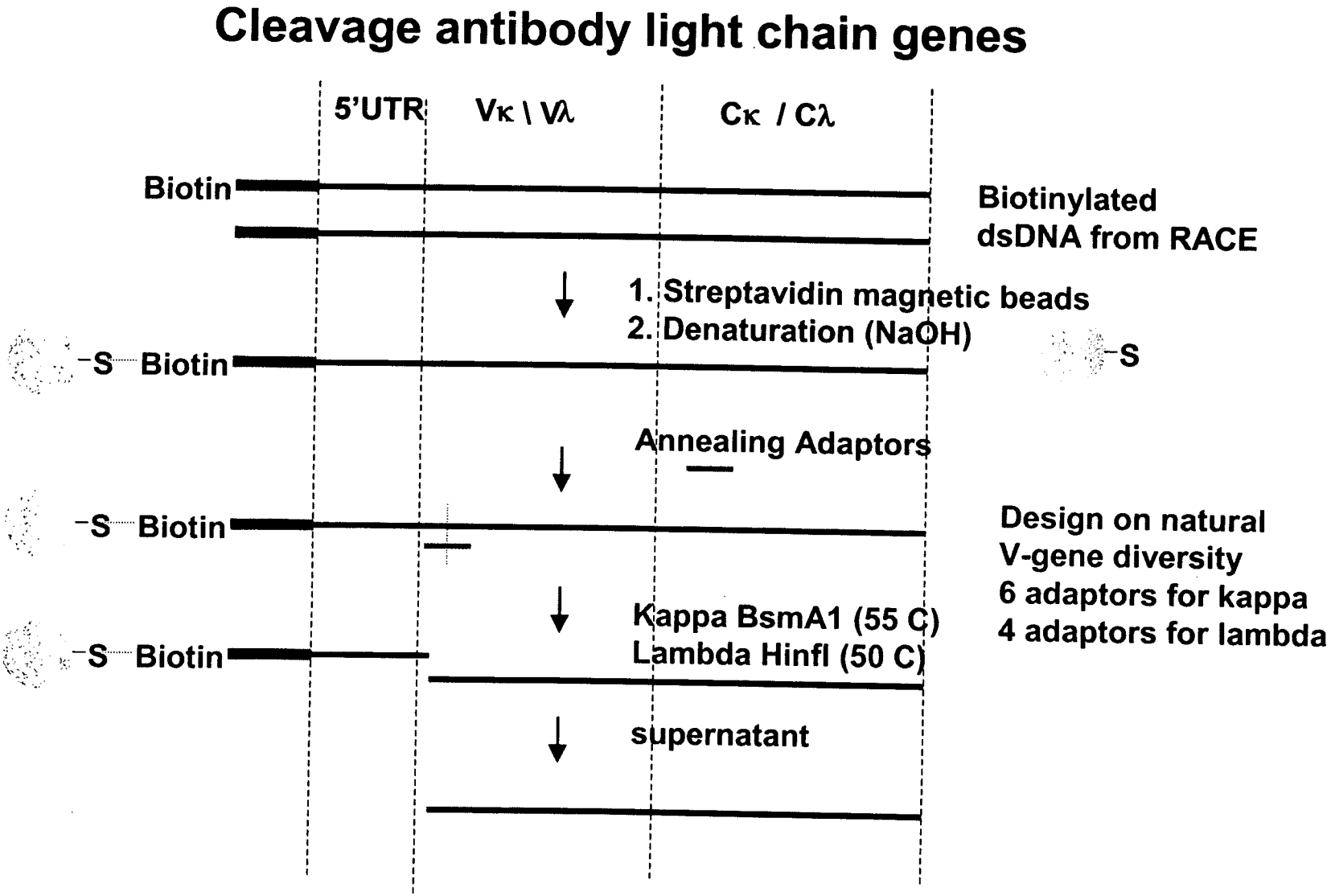

Cleavage antibody light chain genes
5'UTR
Vκ \ Vλ
Cκ / Cλ
Biotin
Biotinylated
dsDNA from RACE
1. Streptavidin magnetic beads
2. Denaturation (NaOH)
S
Biotin
Annealing Adaptors
Design on natural
V-gene diversity
6 adaptors for kappa
4 adaptors for lambda
Kappa BsmA1 (55 C)
Lambda HinfI (50 C)
supernatant

FIG. 12A

Ligation of cleaved light chains

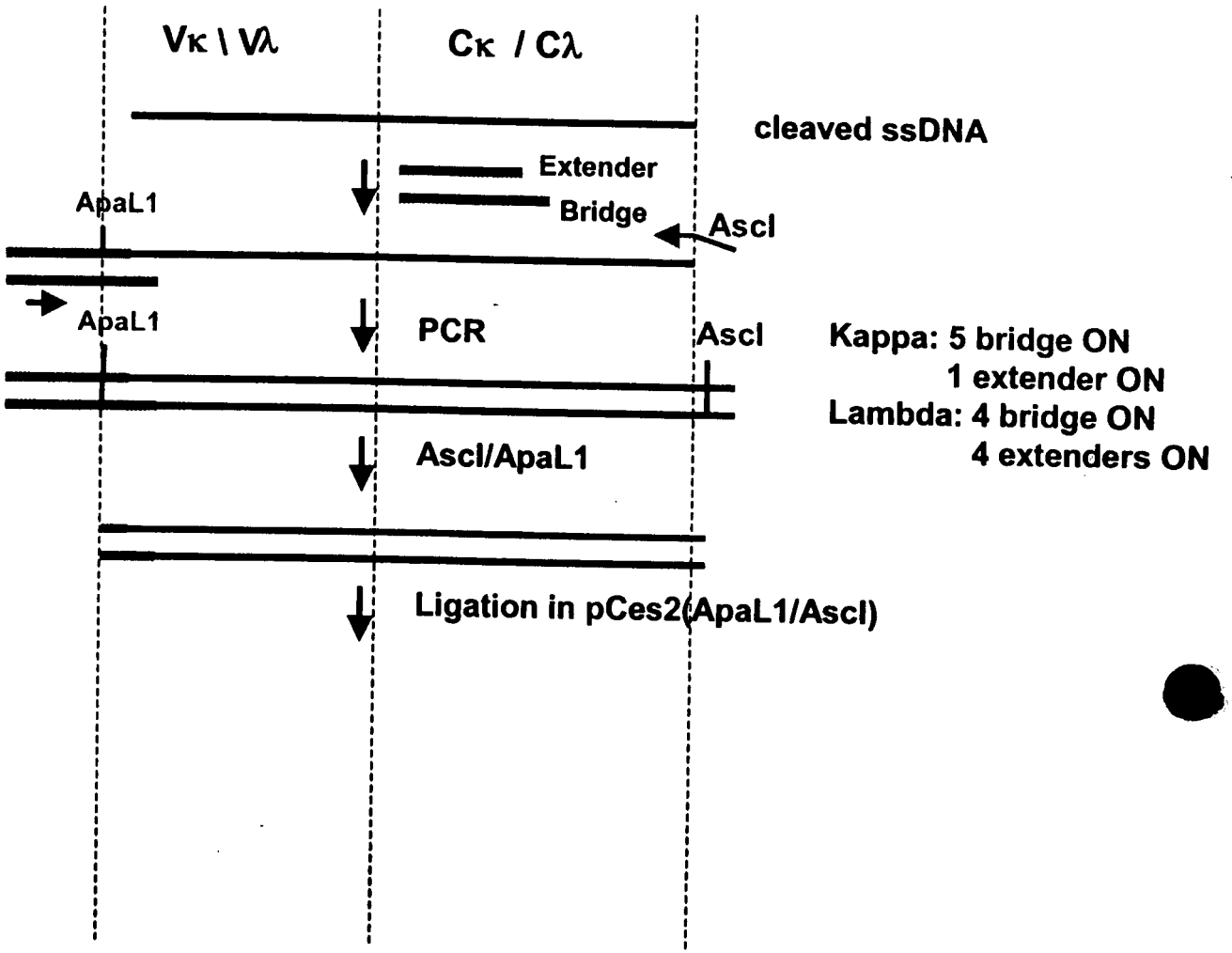
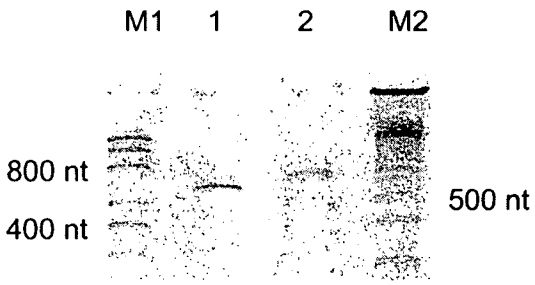


FIG. 12B

Figure 3: Cleavage and ligation lambda light chains

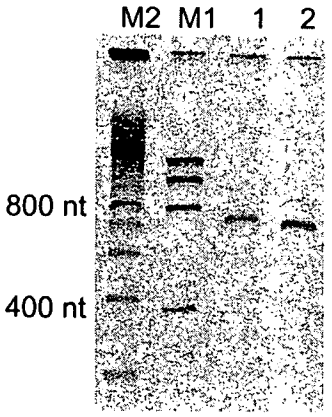
A) HinfI cleavage



M1 = LDM MW marker
1 = sup. after Hinf-cleavage (10ng ssDNA)
2 = beads after Hinf-cleavage (10ng ssDNA (not cleaved)
M2 = 100bp marker , bottom to top: 300nt, 400nt, 500nt, 600nt, 800nt

70% cleavage

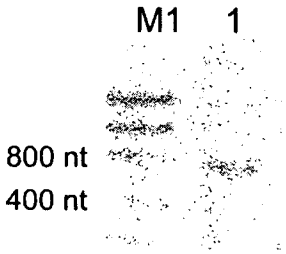
B) Bridge ligation



1=Ligated DNA
2= Unligated DNA

95% ligation

C) PCR



1= 1ul purified PCR product / 50 ul (15 cycles)

FIG. 13

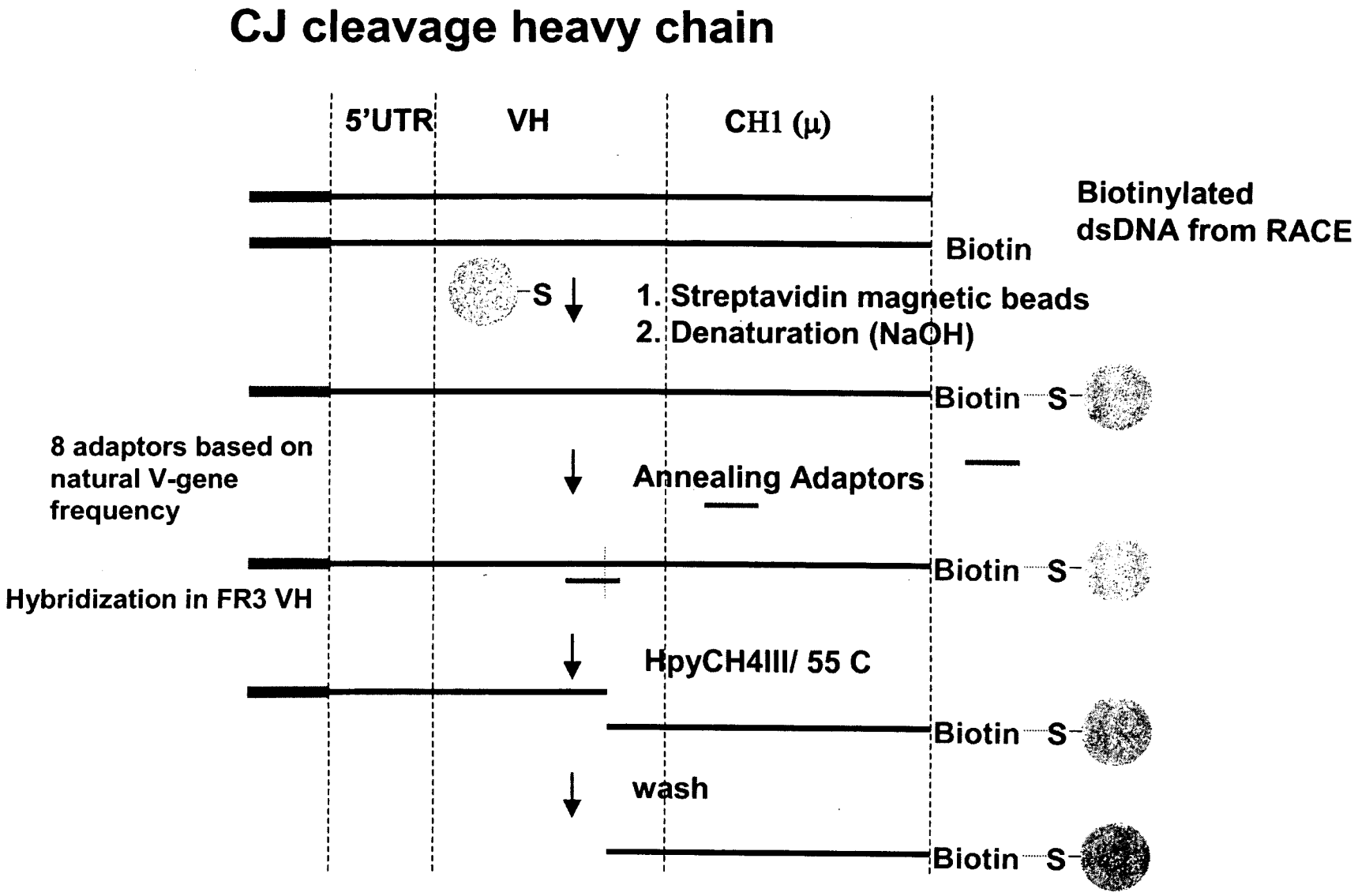

CJ cleavage heavy chain
5'UTR
VH
CH1 (μ)
Biotinylated dsDNA from RACE
Biotin
S
1. Streptavidin magnetic beads
2. Denaturation (NaOH)
Biotin S
8 adaptors based on natural V-gene frequency
Annealing Adaptors
Biotin S
Hybridization in FR3 VH
HpyCH4III/ 55 C
Biotin S
wash
Biotin S

FIG.14A

Ligation heavy chain CDR3 diversity

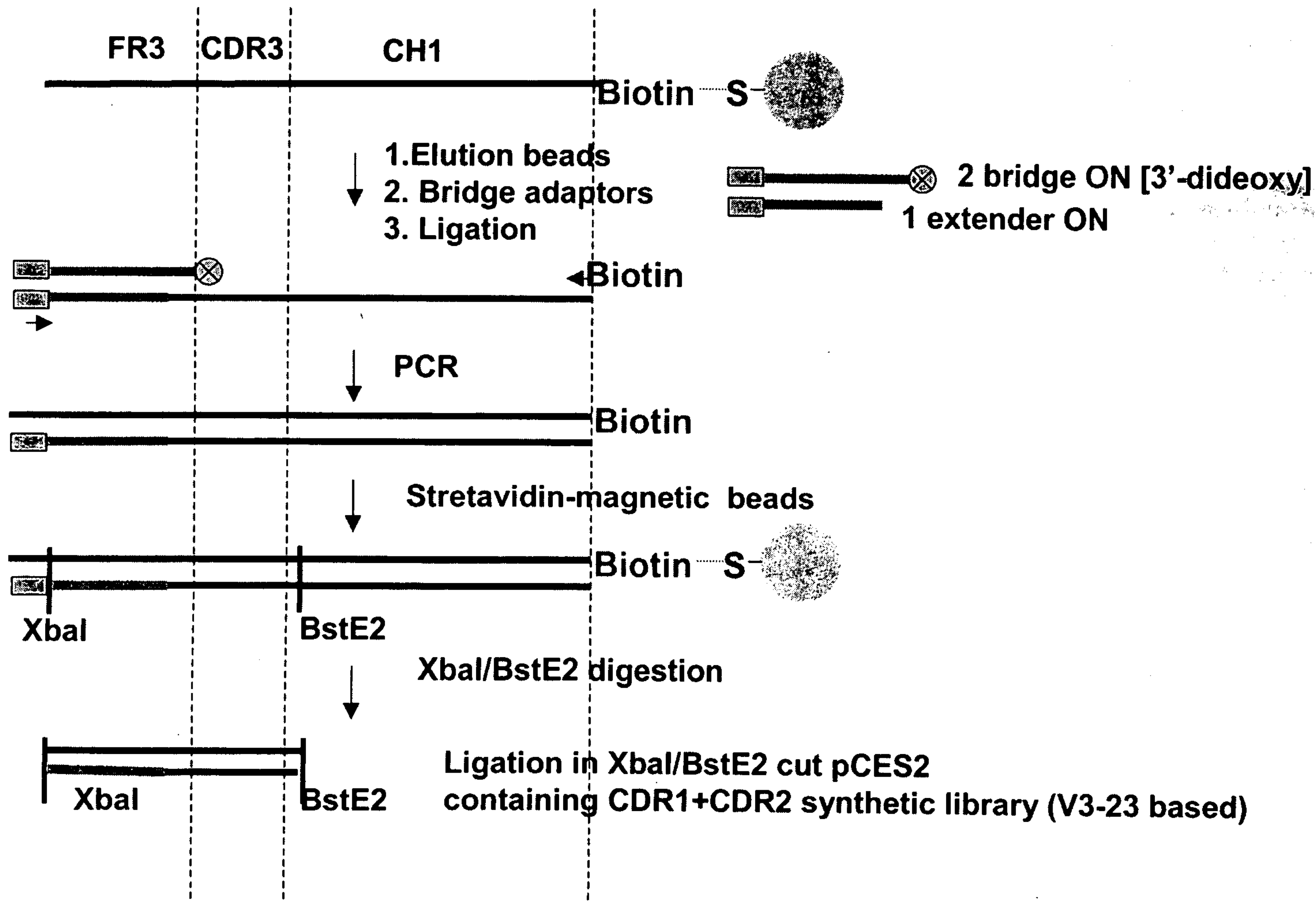
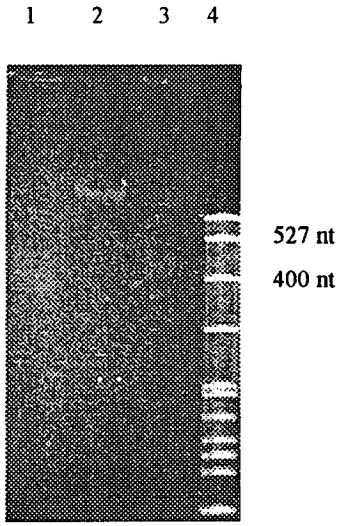


FIG. 14B

Cleavage and ligation Heavy Chain

A) HpyCH4III cleavage



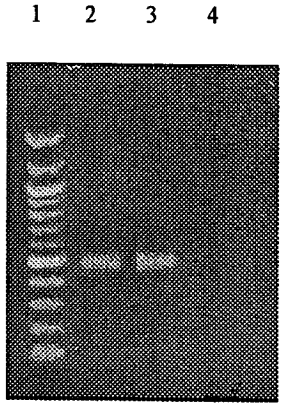
1 = Cleaved DNA eluted from PN column

2 = Beads after HpyCH4III digestion

3 = Supernatant after cleavage

4 = MspI digest of pBR322

B) PCR



1 = NEB 100bp ladder

2 = 5ul/100ul PCR product 20 cycles; sample A

3 = 5ul/100ul PCR product 20 cycles; sample B

4 = no template

FIG. 15

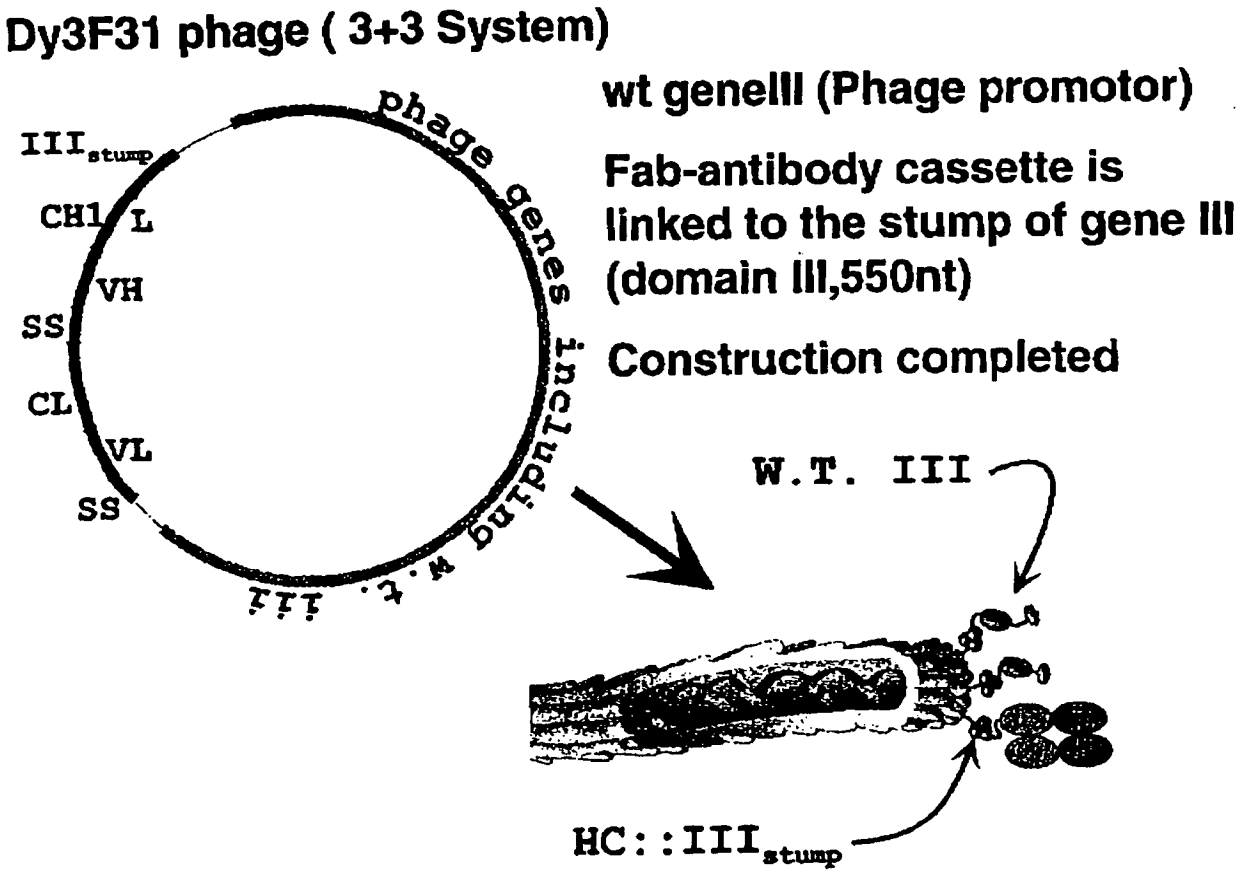
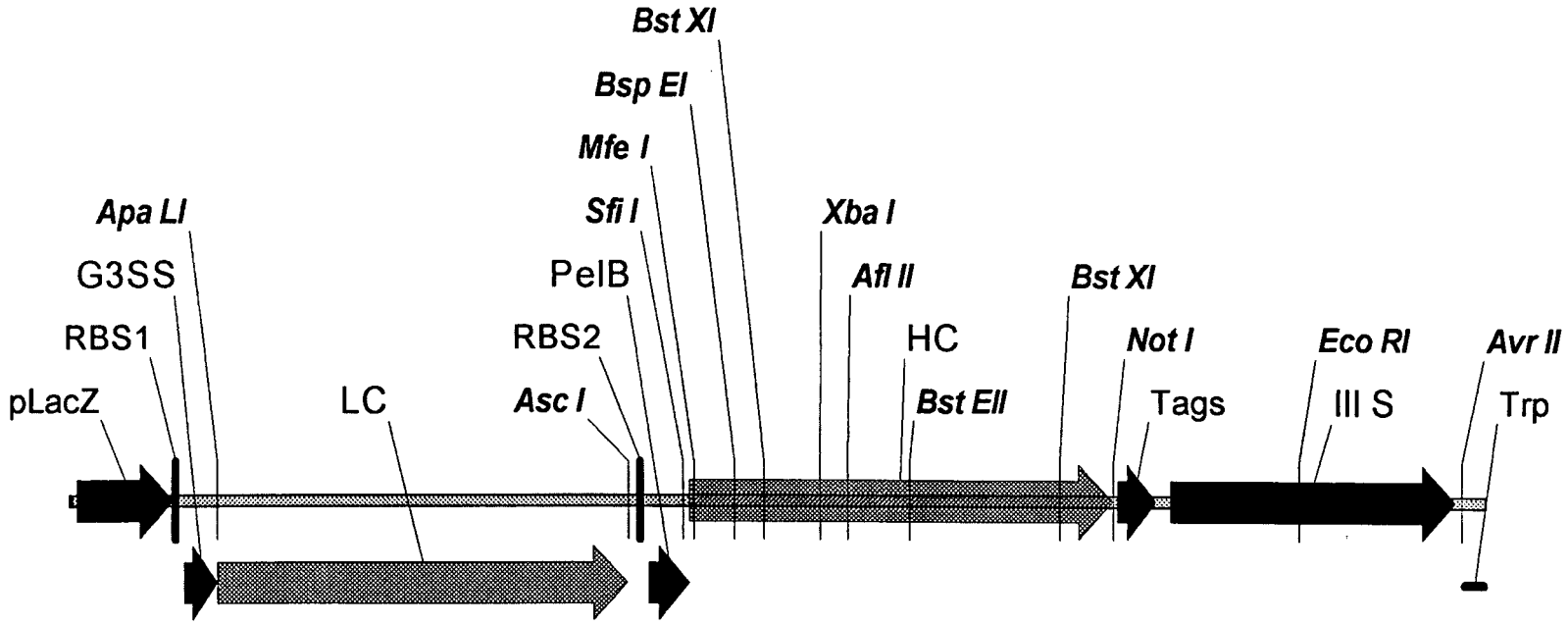


FIG. 16



Fab Cassette

2263 bp

FIG. 17

FIG. 18

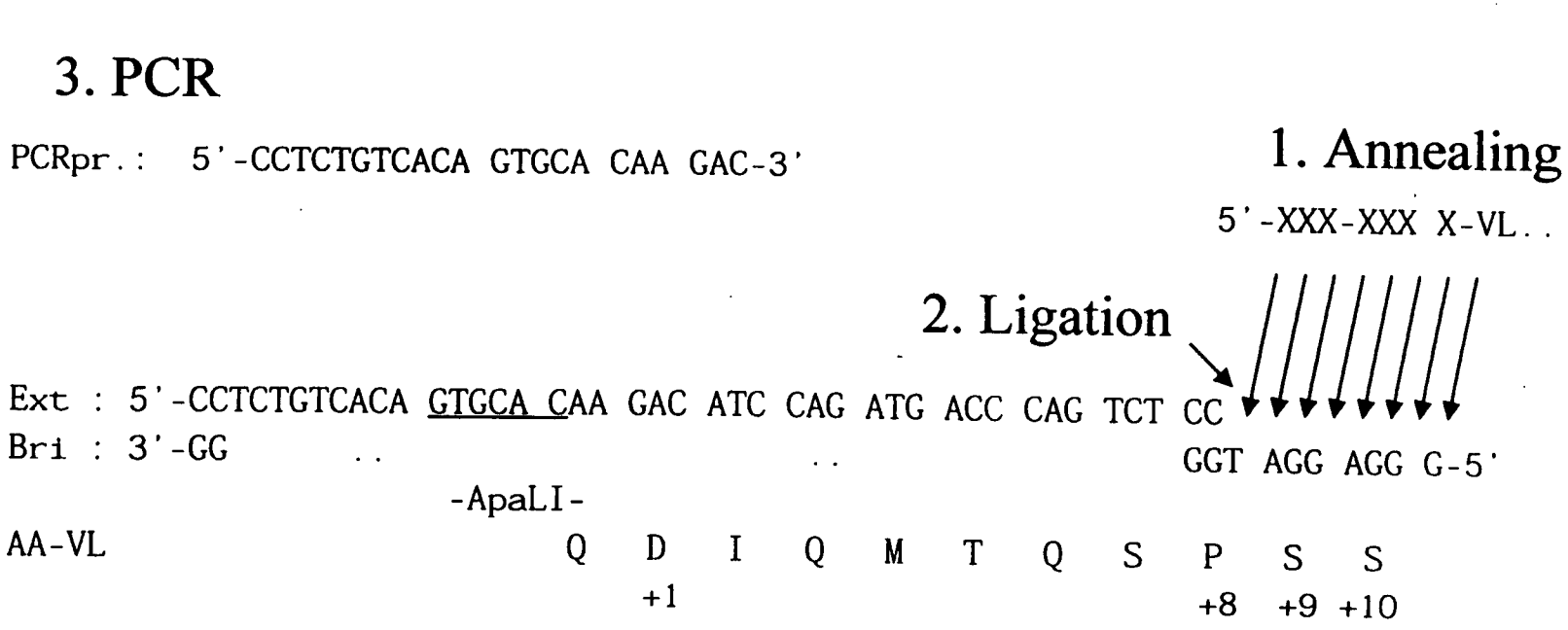

3. PCR
PCRpr.: 5'-CCTCTGTCACA GTGCA CAA GAC-3'
1. Annealing
5'-XXX-XXX X-VL..
2. Ligation
Ext : 5'-CCTCTGTCACA GTGCA CAA GAC ATC CAG ATG ACC CAG TCT CC
Bri : 3'-GG .. .. GGT AGG AGG G-5'
-ApaLI-
AA-VL Q D I Q M T Q S P S S
+1 +8 +9 +10

FIG. 19

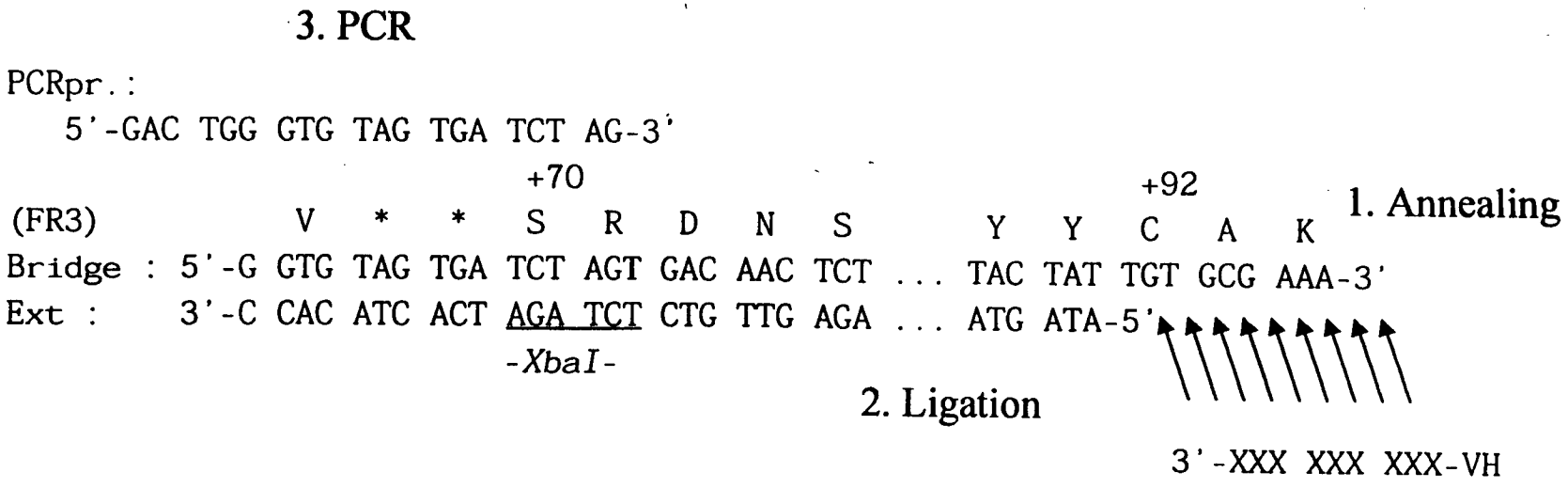


FIG. 20